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Authors

Liao, Hui-Ling
Bonito, Gregory
Rojas, J Alejandro
et al.

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Title: Fungal endophytes of *Populus trichocarpa* alter host phenotype, gene expression and rhizobiome composition

Authors: Hui-Ling Liao¹, Gregory Bonito², J. Alejandro Rojas^{3,4}, Khalid Hameed³, Steven Wu⁵, Christopher W. Schadt⁶, Jessy Labbé⁶, Gerald A. Tuskan⁶, Francis Martin⁷, Igor V. Grigoriev⁸ and Rytas Vilgalys³.

Affiliations: ¹University of Florida, ²Michigan State University, ³Duke University, ⁴University of Arkansas, ⁵Independent Researcher, ⁶Oak Ridge National Laboratory, ⁷INRA, ⁸US Department of Energy Joint Genome Institute and Department of Plant and Microbial Biology, University of California Berkeley

Addresses: ¹North Florida Research and Education Center/University of Florida 155 Research Road, Quincy, FL 32351; ²Michigan State University, Plant Soil and Microbial Sciences, East Lansing, MI, USA 48824; ³Department of Biology Durham, NC, USA; ⁴ 211 PTSC-Fayetteville, AR 72701, ⁵Independent Researcher, Davis, USA; ⁶1 Bethel Valley Road, Oak Ridge, TN 37830; ⁷INRA, UMR 1136 INRA-University of Lorraine, Interactions Arbres/Microorganismes, Laboratory of Excellence ARBRE, INRA-Nancy, 54280, Champenoux, France ⁸DOE Joint Genome Institute, 2800 Mitchell

22 Drive, Walnut Creek CA 94598

23 **Corresponding author:** Hui-Ling Liao¹, sunny.liao@ufl.edu

24

25 **Authors' contribution:**

26 Experiments designed and performed by: H.-L.L., G.B., K.H., C.S., J.L.,
27 G.T. and R.V.

28 Data assembly and analysis: H.-L.L. S.W. A R-F and G.B.

29 Genome sequencing support: I.G. and F.M.

30 Paper writing: H.-L.L, G.B. & R.V. All of the authors contributed to
31 manuscript editing.

32 The authors declare no conflict of interest

33

34

35 **Abstract:**

36 *Mortierella* and *Ilyonectria* include common species of soil fungi which are
 37 frequently detected as root endophytes in many plants including *Populus*
 38 spp. However, the ecological roles of these and other endophytic fungi with
 39 respect to plant growth and function are still not well understood. The
 40 functional ecology of two key taxa from the *Populus* rhizobiome, *Mortierella*
 41 *elongata* PMI93 and *Ilyonectria europaea* PMI82, was studied by coupling
 42 forest soil bioassays with environmental metatranscriptomics. Using soil
 43 bioassay experiments amended with fungal inoculants, *M. elongata* was
 44 observed to promote the growth of *Populus*. This response was cultivar
 45 independent. In contrast, *I. europaea* had no visible effect on *Populus*
 46 growth. Metatranscriptomic studies reveal that these fungi impact
 47 rhizophytic and endophytic activities in *Populus* and induce shifts in soil and
 48 root microbial communities. Differential expression of core genes in *P.*
 49 *trichocarpa* roots was observed in response to both fungal species.
 50 Expression of *Populus* genes for lipid signaling and nutrient uptake were up-
 51 regulated and expression of genes associated with gibberellin signaling were
 52 altered in plants inoculated with *M. elongata*, but not *I. europaea*. Up-
 53 regulation of genes for growth promotion, down-regulation of genes for
 54 several LRR-receptors/kinases, and alteration of expression of genes
 55 associated with plant defense responses (e.g., JA/ET/SA pathways) also
 56 suggest that *M. elongata* manipulates plant defenses while promoting plant
 57 growth.

58 **Introduction**

59 Soils of *Populus* and forest trees harbor a high diversity of rhizospheric
60 fungi with diverse ecological functions, including mycorrhizal fungi,
61 endophytes, saprophytes and pathogens (Bonito, et al. 2016). In particular,
62 *Populus* species associate with a high diversity of root endophytes which play
63 key roles in rhizosphere function and plant fitness (Shakya et al. 2013;
64 Cregger et al. 2018). While several *Populus*-ectomycorrhizal interactions
65 have been reported (Lodge 1989; Podila et al. 2009; Baum and Makeschin
66 2000; Bois et al. 2005; Gottel et al. 2011; Guevara et al. 2013; Martin et al.
67 2016), the mechanisms involved in *Populus*-endophytes interactions that
68 affect plant growth and fitness remain unexplored.

69 Recent studies have identified *Mortierella* spp. as part of the core
70 *Populus* microbiome (Gottel et al. 2011; Shakya et al., 2013; Bonito et al.
71 2014; Uehling et al. 2017). *Mortierella* belongs to Mucoromycota, an early
72 diverging phylum of fungi, which is comprised of Glomeromycotina
73 (arbuscular mycorrhizal fungi), Mortierellomycotina and Mucoromycotina
74 (Bidartondo et al. 2011; Spatafora et al. 2016; Strullu-Derrien et al. 2018).
75 Most *Mortierella* spp. are considered to be soil saprophytes, however they
76 are also frequently isolated as endophytes from surface sterilized healthy
77 root tissue of *Populus* and other plant species (Bonito et al. 2016). Beneficial
78 interactions between *Mortierella* and plants are known to exist, but
79 functional and mechanistic studies on plant-*Mortierella* interactions are few.

80 A recent study showed that *Mortierella hyalina* enhanced aboveground
 81 biomass of *Arabidopsis* and activated host Ca^{2+} signaling to suppress
 82 immune responses (Johnson et al. 2018). Another study showed the ability of
 83 endophytic *Mortierella alpina* to enhance the stress tolerance in host plants
 84 as a root endophyte via biosynthesis of the tetraterpenoid-derived
 85 phytohormones *in planta*, including apocarotenoid (Wani et al. 2017).
 86 Genome analysis and carbon utilization assays suggest that *M. elongata*
 87 metabolism is largely based on simple carbon utilization (e.g., D-glucose, D-
 88 trehalose and D-mannose) and that its metabolism is enriched in lipids and
 89 polyunsaturated fatty acids (PUFAs) anabolism (Uehling et al. 2017). Based
 90 on their enzyme profile, *Mortierella* spp. can acquire organic nitrogen
 91 through chitinolytic activities (Uehling et al. 2017; Vadivelan and
 92 Venkateswaran 2014) utilizing the chitin monomer N-acetyl glucosamine as a
 93 nitrogen and carbon source.

94 *Ilyonectria* spp. are another common group of ubiquitous rhizosphere
 95 fungi whose function as endophytes is poorly known. *Ilyonectria* belongs to
 96 the family of Nectriaceae (Hypocreales, Sordariomyceta, Ascomycota),
 97 however, the taxonomy status of *Ilyonectria* and other related genera is still
 98 in flux. For instance, molecular systematic studies revealed a high amount of
 99 cryptic speciation within the *Ilyonectria* complex (Cabral et al. 2012).
 100 *Ilyonectria* spp. are commonly isolated from rhizosphere soils and as
 101 endophytes from surface sterilized healthy roots from a wide range of woody
 102 and herbaceous plants including *Populus* (Cui et al. 2015; Kwaśna et al.

2016). *Ilyonectria* species are generally assumed to be commensals or weak plant pathogens, since some species are associated with disease of certain plant hosts, including root rot in grapes (Cabral et al. 2012; Hersh et al. 2012) and ginseng (Farh et al. 2018). However, as with *Mortierella*, the ecological function of most *Ilyonectria* species is unknown.

We hypothesize signaling between *Populus* and other non-mycorrhizal fungal root endophytes can occur in a community context and is bidirectional, such that each symbiont impacts the transcriptional regulation of its partner. However, we predict that *P. trichocarpa* uses different strategies to interact with *Mortierella* compared to *Ilyonectria*, given these fungal taxa are separated by a large phylogenetic distance and are assumed to represent opposite ends of the ‘pathogen-mutualist’ ecological spectrum. We carried out bioassay experiments with *P. trichocarpa* to test our hypotheses that *M. elongata* (PMI93) and *I. europaea* (PMI82) elicit different molecular responses from their native host *Populus trichocarpa*. We used RNA-Seq to investigate the functional activities of these fungal generalists (PMI93 and PMI82) in the rhizosphere and to identify the key plant genes responsive to fungal inoculation. This study highlights how different functional groups of endophytic fungi interact with a single host plant, *P. trichocarpa* and provides new insights into the relationships between species coexistence, plant fitness and ecosystem functions.

Results

125 *Mortierella elongata* (PMI93) promotes plant growth

126 To test if endophytic fungal taxa belonging to the core *Populus*
 127 rhizobiome contribute to plant fitness, we used bioassay experiments to
 128 examine the response of *Populus* to inoculation with two fungi isolated from
 129 *Populus* roots, *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82)
 130 (Bonito *et al.*, 2016). *Populus* cuttings were grown in a background of soils
 131 collected from *Populus* sites in North Carolina, USA and inoculated with *M.*
 132 *elongata* (PMI93) or *I. europaea* (PMI82), respectively. *M. elongata* (PMI93)
 133 enhanced whole plant dry weight (30%, $p \leq 0.05$), and leaf expansion
 134 ($p \leq 0.05$). Additionally we observed an increased amount of chloroplasts in
 135 *Populus* (Fig. 1; Supplementary Fig. 1). Particularly, *M. elongata* (PMI93)
 136 enhanced the dry weight in *Populus* roots more than it did in aboveground
 137 organs (Fig. 1). While *Populus* growing in both soil and sand, *M. elongata*
 138 (PMI93)-triggered-growth of aboveground organs was not plant genotype-
 139 dependent (Supplementary Fig. 1). In contrast, *I. europaea* (PMI82) promoted
 140 *Populus* growth in sterilized sand, but otherwise had no effect on plant
 141 growth (Fig. 1B). The response of *Populus* to *I. europaea* was investigated
 142 only on a single genotype, BESC4 (Fig. 1B), we cannot exclude a positive or
 143 negative response with other genotypes.

144 *Fungi as biotic factors that influence the composition of fungal*
 145 *communities in Populus roots and soils*

RNA-Seq data indicate that there were populations of *Mortierella* and *Ilyonectria* in the soils used for these bioassays (Supplementary Dataset 1; Fig. 2), however, inoculation resulted in a larger population of the target taxa and a higher relative abundance of target mRNA recovered (Fig. 2A). The inoculation with *M. elongata* (PMI93) increases the target fungus only in soil samples, whereas that with *I. europaea* (PMI82) in both root and soil samples (Fig. 2A). *M. elongata* (PMI93) inoculation resulted in an increase of relative abundance of *Ilyonectria* mRNA in the root and soil (Fig. 2B). *I. europaea* (PMI82) inoculation resulted in an increase of relative abundance of *Mortierella* mRNA in the soil (Fig. 2B).

To explore the interactive effects of *M. elongata* (PMI93) and *I. europaea* (PMI82) on the fungal community in *Populus* roots and soils, fungal LSU rRNA reads (corresponding to divergent domains D1 and D2) were extracted from the RNA-Seq data and used to identify fungal community composition in soil and roots as described by Liao *et al.* (2014). Species-rich communities of root-associated fungi were detected in individual *Populus* roots and rhizosphere soils (Fig. 3). A high diversity of fungi across different ecological guilds, from mutualists to pathogens, were present in all samples. A block effect was observed (Fig. 3), whereby the microbial community structure of uninoculated *M. elongata* (PMI93) samples were more similar to inoculated *M. elongata* (PMI93) samples and the microbial community structure of uninoculated *I. europaea* (PMI82) samples were more similar to *I. europaea* (PMI82) inoculated samples, likely explained by the fact that two

169 different soils (harboring different microbial communities) were used for the
 170 two separate experiments. Therefore, the two experiments were analyzed
 171 independently. Further, higher variability of soil and root fungal community
 172 structure was found in the *I. europaea* (PMI82) experimental samples
 173 compared to *M. elongata* (PMI93) experimental samples, regardless of the
 174 addition of fungal inoculum (Supplementary Fig. 3; Supplementary Table 2).
 175 Over 90% of the detected fungi are root associated fungi, including
 176 arbuscular (AMF) and ectomycorrhiza fungi (EMF), endomycorrhizal fungi and
 177 fungal endophytes. It is not known if physiological conditions of *Populus*
 178 cuttings used for the individual replicates contributed to the variability of
 179 fungal microbiomes. The physiological conditions of plants were not
 180 examined other than the measurement of plant biomass (Fig. 1). In addition,
 181 inoculation with either *M. elongata* (PMI93) or *I. europaea* (PMI82) resulted in
 182 a shift in the composition of the fungal soil community compared to
 183 uninoculated plants grown in the same soils ($p \leq 0.05$) (Supplementary Fig.
 184 3A; Supplementary Table 2A). AMF transcriptome activity changed
 185 significantly in the roots and soil inoculated with *I. europaea* (PMI82), but not
 186 with *M. elongata* (PMI93) ($p \leq 0.05$; Supplementary Fig. 3B1). *M. elongata*
 187 (PMI93) inoculations reduced some AMF taxa in root tissues, including those
 188 of Glomeromycota, *Septoglomus* and *Scutellospora* (Fig. 3; Supplementary
 189 Table 2C). Soil inoculation with *M. elongata* (PMI93) resulted in increased
 190 transcriptome activity by EMF (Supplementary Fig. 3B2 and 3B3), an effect
 191 that has also been observed for other fungi in the *Nectriaceae* (Swett and

Gordon 2016). The transcriptome composition of endophytes in the soils was shifted in response to both *M. elongata* (PMI93) and *I. europaea* (PMI82) inoculation (Supplementary Fig. 3B4 and 3B5). Inoculation with *M. elongata* (PMI93) resulted in enrichment of certain fungal endophytes (*Gibberella*, *Bionectria*, *Neonectria*, *Neocosmospora*, *Nectria* and *Trichoderma*) in both root and soil systems (Fig. 3). *I. europaea* (PMI82) inoculation resulted in the enrichment for the fungal pathogens, *Leptosphaerulina* and *Didymella* (Fig. 3; Supplementary Fig. 3B6; Supplementary Table 2C). Finally, inoculation with *M. elongata* (PMI93) and *I. europaea* (PMI82) resulted in a shift in the composition of transcribed rRNA of saprotrophs (Supplementary Fig. 3B7).

Shared and unique responses of Populus responses to Mortierella elongata (PMI93) and Ilyonectria europaea (PMI82) inoculations

We compared the net transcriptomic activity of *Populus* roots with and without fungal inoculation. We recovered ~34 million reads from individual pools of fine roots (around 1 mg; Supplementary Dataset 1). The average read proportion of expressed genes was 72%:0.1%:28 %, (plant: inoculated fungi: other species not mapped to plant hosts or inoculated fungal genomes) for individual samples regardless of whether the samples were inoculated with fungi (Fig. 2; Supplementary Dataset 1). Additional details of computational pipeline used for data assemblies can be found in Supplementary Fig. 2.

214 Inoculation with *M. elongata* (PMI93) and *I. europaea* (PMI82) resulted
 215 in a strong molecular plant response (Fig. 4; Supplementary Dataset 2). Over
 216 4,497 individual genes of *Populus* were significantly altered in response to *M.*
 217 *elongata* (PMI93) inoculation ($\text{FDR} \leq 0.05$; ≥ 2 -fold changes; $n=4$) (Fig. 4A). In
 218 contrast, only 380 genes of *Populus* changed their expression patterns in
 219 response to inoculation with *I. europaea* (PMI82) (Fig. 4A). Further, replicate
 220 samples of *I. europaea* (PMI82) appear to be much more variable than for *M.*
 221 *elongata* (PMI93) (Fig. 3 and 5). Likely, the higher variability in gene
 222 expression across replicates is the cause of fewer differentially expressed
 223 *Populus* genes in response to *I. europaea* (PMI82) compared to *M. elongata*
 224 (PMI93) inoculation.

225 Expression patterns of 260 genes changed in *Populus* in response to both
 226 *M. elongata* (PMI93) and *I. europaea* (PMI82) (shared genes) (Fig. 5;
 227 Supplementary Dataset 3), indicative of molecular commonalities in how
 228 *Populus* responds to different species of rhizosphere fungi. Pathways
 229 mediating this shared response include carbohydrate metabolism, plant cell
 230 wall development, fatty acid/lipid biosynthesis and metabolisms, IAA
 231 signaling, heat shock, stress response and transport and intracellular
 232 signaling/transcriptional regulation. As part of this common response, a few
 233 functional groups, including several plant defense related genes (18 genes),
 234 are significantly up- and down- regulated ($\text{FDR} \leq 0.05$; ≥ 2 -fold changes; $n=4$).
 235 These include JA/ET/ABA biosynthesis and signaling and salicylic acid (SA)-
 236 LRR-mediated signaling. For example, one gene for JA signaling (12-

237 oxophytodienoate reductase) was up-regulated (Gene ID: Potri.013G102700)
 238 and two genes encoding lipoxygenase were down-regulated
 239 (Potri.005G032700; Potri.005G032400) (Supplementary Table 1;
 240 Supplementary Dataset 3). The NDR1/NIH1-like gene (Potri.017G154000),
 241 which has been reported to respond to SA-mediated biotic stress, was also
 242 up-regulated in response to fungal inoculation (Wu et al. 2012).

243 Aside from shared genes, 4,237 and 120 *Populus* genes were predicted
 244 to respond to *M. elongata* (PMI93) and *I. europaea* (PMI82), respectively.
 245 *Populus* responded more strongly to *M. elongata* (PMI93) compared to *I.*
 246 *europaea* (PMI82) at physiological and molecular levels. Thus, further
 247 analyses focused specifically on *Populus* responses to *M. elongata* (PMI93).
 248 The majority of functional groups of these unshared genes are involved
 249 within transmembrane functions (32% of total unshared genes), extracellular
 250 functions (7%) and transcriptional regulators (8%) regardless of soil batch or
 251 fungal species was used as inoculum (Supplementary Fig. 4). Of 356 plant
 252 genes involved in extracellular activities during *Populus-M. elongata* (PMI93)
 253 interaction, 147 genes (41% of extracellular proteins) were considered to be
 254 plant small secreted proteins (pSSPs), comprising up to 8% total pSSPs
 255 (1,680 pSSPs) from *P. trichocarpa* genome (Tuskan et al. 2006; Yang et al.
 256 2011). Of 147 *Populus* pSSPs, 94 were up-regulated and 53 genes were
 257 down-regulated in response to *M. elongata* (PMI93) (Fig. 6; Supplementary
 258 Dataset 4). Genes encoding pSSPs involved in plant lipid-transfer proteins
 259 (pLTPs) and cell wall loosening (expansin) were up-regulated, while genes

260 encoding pSSPs related to cell adhesion and plant defense response were
 261 down-regulated (Gene IDs were shown in column A and the predicted
 262 functions were shown in column CG of Supplementary Dataset S4A).

263 Of 85 plant genes involved in extracellular activities during *Populus- I.*
 264 *europaea* (PMI82) interaction, 15 genes (18%) were predicted to be pSSPs
 265 (Supplementary Dataset 4B). One gene encoding an pSSPs for pLTPs
 266 (Potri.013G131500), two genes for protein app1 and three genes for cell wall
 267 protein gp1-like were up-regulated. Other genes encoding pSSPs, including
 268 serine protease inhibitor (Kazal-type), clavata3 and plant natriuretic peptide
 269 A, were down-regulated. None of those 15 genes encode for cell adhesion
 270 and plant defense response. The protein structure analysis were furthered
 271 applied to study the structural architectures of those pSSP groups
 272 (Supplementary Fig. 5).

273 Two distinct pLTPs families (Family 1 LTPs; Family 2 LTPs) have been
 274 biochemically characterized (Yeats and Rose 2008). All 12 *Populus* pLTPs
 275 identified in response to *M. elongata* (PMI93) share a structural architecture
 276 of a hydrophobic cavity enclosed by four alpha-helices that are folded using
 277 four disulfide bounds (Supplementary Fig. 5A). The conserved eight-cysteine
 278 motif contributes to these four disulfide bounds. The presence of Tyrosine-16
 279 and the small hydrophobic amino acid (Ile, Val, Leu, Ala) direct *Populus*
 280 pLTPs as the Family 1 pLTPs. These *Populus* pLTPs vary in amino acid identity
 281 (between 1%-90% identity).

282 *Populus* pSSPs associated with defense responses were predominantly
 283 down-regulated in response to *M. elongata* (PMI93) including three groups:
 284 dirigent-like protein, germin-like protein and PR-thaumatin associated protein
 285 (Fig. 6; Supplementary Fig. 5B; Supplementary Dataset 4). Ligand and
 286 enzyme activity prediction analysis showed different modes of the plant
 287 immune systems, including JA/ET/SA/ABA mediated pathways, were
 288 suppressed in association with *M. elongata* (PMI93) inoculation. Along this
 289 line, although one gene for JA signaling (12-oxophytodienoate,
 290 Potri.013G102700) was up-regulated in *Populus* in response to both *M.*
 291 *elongata* and *I. europaea* inoculation, other 12-oxophytodienoate reductases
 292 (Potri.003G004600 and Potri.003G004200) were conversely downregulated
 293 in the root inoculated by *M. elongata* (PMI93). All detected genes for
 294 lipoxygenases (Potri.005G032400, Potri.005G032700, Potri.005G032600,
 295 Potri.013G022100, Potri.009G022400) (Supplementary dataset 1) were also
 296 downregulated in *M. elongata* (PMI93) inoculated roots. Expression of 23
 297 *Populus* genes for gibberellin signaling was altered in plants inoculated with
 298 *M. elongata*, but not *I. europaea* (Supplementary Table 1B).

299 We further categorized these genes through different annotation
 300 methods, including KEGG mapper (Supplementary Fig. 6), KOG gene groups
 301 (JGI annotation) (Supplementary Fig. 7) and ClueGO gene enrichment
 302 analysis (Bindea et al. 2009;2013) (Supplementary Fig. 8). Results of these
 303 analyses indicate that *M. elongata* (PMI93) inoculation contributed to an up-
 304 regulation in *Populus* pathways involved in fatty acid/glycerolipid

305 biosynthetic processes and metabolism, and oxidative phosphorylation.
 306 Conversely, there was a down-regulation of genes involved in carotenoid
 307 biosynthesis and ET/JA/SA signaling ($p \leq 0.05$; ≥ 2 -fold changes; $n=4$). The
 308 most abundant differentially expressed genes appear to be mostly involved
 309 in signaling (Fig. 5; Fig. 6; Supplementary Dataset 2A), including receptor
 310 kinases and transcription factors. A majority of receptor kinases were down-
 311 regulated in response to *M. elongata* (PMI93) including 61 genes for LRR-
 312 receptor kinases (LRR-RKs) (Supplementary Dataset 2A). ClueGo gene
 313 enrichment analysis also shows that inoculation with *M. elongata* (PMI93)
 314 enhances the activities of fatty acid biosynthesis, thiolester hydrolase,
 315 response to inorganic substrates, cytokinin metabolism and disaccharide
 316 biosynthesis (Supplementary Fig. 8B).

317 Of 120 genes in *Populus* that responded to *I. europaea* (PMI82)
 318 inoculation, but not *M. elongata* (PMI93) inoculation, two genes were
 319 predicted to be pLRRs (Potri.005G043700; Potri.019G110800) and were
 320 down-regulated (Supplementary Dataset S2B).

321 *Fungal genes up-regulated in response to Populus*

322 Our initial attempts to profile the expression pattern of *M. elongata*
 323 (PMI93) and *I. europaea* (PMI82) genes in roots and soils was hampered by a
 324 low abundance of fungal endophyte reads (Fig. 2). For example, only 56K
 325 paired-reads of *M. elongata* (from over 28M qualified reads) were detected
 326 from individual soil samples (Supplementary Dataset 1). This may be a

327 general feature of many endophytes which are characterized by lower
 328 activity and abundance especially within plant tissues. Because *Mortierella*
 329 expressed higher numbers of mRNA transcripts than to *Ilyonectria* for the soil
 330 and root samples without inoculation (Fig. 2), the molecular activities of *M.*
 331 *elongata* (PMI93) in the bioassay were further investigated. The higher
 332 abundance of *M. elongata* (PMI93) mRNA transcripts in rhizosphere soil (Fig.
 333 2) permitted us to compare relative expression comparisons of fungal genes
 334 (% reads of *M. elongata* = 5%, around 1.4 M reads per sand sample)
 335 (Supplementary Dataset 1). In total, 7,950 genes of *M. elongata* (PMI93)
 336 were detected across all (4) biological replicates of sand samples inoculated
 337 with *M. elongata* (PMI93). To study the functional categories active in *M.*
 338 *elongata* (PMI93), we investigated the number of transcribed genes and their
 339 functional proportions of *M. elongata* (PMI93) detected in sand and *in vitro*
 340 (Supplementary Fig. 9; Supplementary Dataset 6). In general, a similar
 341 pattern and proportion of *M. elongata* (PMI93) functional genes was detected
 342 across sand and culture conditions. However, fewer *Mortierella* genes
 343 encoding secreted proteins (fSSPs), leucine-rich receptors and WD40 were
 344 detected in *Populus*-sand bioassay treatments inoculated by *M. elongata*
 345 (PMI93), compared to when *M. elongata* (PMI93) was grown in pure culture
 346 axenically ($p \leq 0.05$) (Supplementary Dataset 6). Of 87 fSSPs detected in the
 347 sand with *Populus* grown nearby, only 3 fSSPs were not detected in the
 348 culture (Supplementary Dataset 7). Comparative metatranscriptomics also
 349 show genes for RNA modification, translation, signal transduction, lipid

transport and metabolism and chitinase were significantly up-regulated in *M. elongata* (PMI93) when *M. elongata* (PMI93) grew with *Populus* compared to pure culture (Supplementary Fig. 9; Supplementary Dataset 7).

Discussion

Some fungal endophytes are known as beneficial symbiotic microbes able to promote plant growth and induce plant defense (Vaarma, et al., 1999; Lee et al., 2011; Zuccaro, et al., 2014; Grelet et al., 2017). In this study, soil inoculation with the ubiquitous fungal endophyte *M. elongata* (PMI93) promoted plant growth, while the effects of inoculation with another common endophyte *I. europaea* (PMI82) were neutral in phenotype (Fig. 1). RNA-Seq data demonstrate that *M. elongata* (PMI93) and *I. europaea* (PMI82) both have a dual lifestyle: each can grow as a root endophyte or as soil saprotroph (Fig. 2). The mycelium of *M. elongata* (PMI93) forms a biofilm on plant roots indicating that *M. elongata* (PMI93) can directly interact with plant roots (Supplementary Fig. 10). The differentiation of an individual fungal mycelial network between two life strategies also implies that a fungal isolate itself may utilize multiple resources, while interacting with plant host(s). Fungal endophytes have been classified into four classes according to their life histories (Rodriguez et al. 2009). *Mortierella* and other Class 4 fungal endophytes live within their host plant roots for at least a part of their life cycle without apparent symptoms (Wilson 1995; Rodriguez and Redman

1997; Rodriguez et al. 2009). In contrast, saprotrophic fungi live off dead organic matter in soils and dead plant tissues. The present of fungal transcriptomes in root and soil suggests that *M. elongata* (PMI93) and *I. europaea* (PMI82) participate in a combination of endophytic and saprotrophic activities (Fig. 2). However, the low proportion of *Mortierella* and *Ilyonectria* transcripts in the soil and root RNA probably reflects the low biomass of this fungus in these two niches. This fact prevented the authors from carrying out a detailed analysis of the *Mortierella* transcriptome in response to a host plant.

Results from this study also demonstrated how enrichment of a single fungal taxon can shift the whole community of root and soil-associated microbes and thus altering the ecological functions of associated plants and diverse soil taxa (Fig. 3). *Mortierella elongata* (PMI93) may promote plant growth indirectly by manipulating the community and functioning of other rhizosphere microbes (Fig. 3), by altering the nutrient composition of soil to facilitate resource acquisition (e.g., nitrogen, lipid) or by modulating plant phytohormones (e.g., IAA, GA) (Supplementary Fig. 6, 7 and 8). The common surveillance genes in *Populus* are activated in response to inoculation of both fungal species (Fig. 5), even though the two fungal species play different roles in associated with *Populus* (Fig. 1). This indicates that *Populus* may react to different biotic conditions through a common set of signaling pathways. Since soils collected destructively over a time interval were used to study the effect of *M. elongata* (PMI93) and *I. europaea* (PMI82)

395 respectively, we cannot exclude the possibility that the different responses
 396 of *Populus* to these two endophytes may be influenced by interactions
 397 between the inoculated endophytes and soil microorganisms.

398 Unlike pathogenic or mycorrhizal fungi that utilize a battery fungal
 399 effectors to modulate plant defenses, the *M. elongata* genome has reduced
 400 amount of fSSPs (417 fSSPs genes in Morel1 genome, Supplementary
 401 Dataset 8) in comparison with fungal specialists or obligate biotrophs
 402 (around 700-1,400 fSSPs) (Kim et al. 2016). We observed similar pattern and
 403 proportion of *M. elongata* (PMI93) fSSPs were present in soil, as compared to
 404 when growing in pure in culture (Supplementary Fig. 9). Thus, direct
 405 interactions between fungal effectors and plant receptors likely play a lesser
 406 role in *Populus-Mortierella* interactions.

407 *Populus* spp. form functional symbioses with both AMF and EMF using
 408 effector-receptor communication (Plett et al. 2011; Martin et al. 2016).
 409 During ectomycorrhizal interaction between *P. trichocarpa* and *Laccaria*
 410 *bicolor*, a fungal effector fSSP (MiSSP7) is taken up by *Populus* and imported
 411 into plant nuclei where it suppresses JA-mediated plant defense response
 412 (Plett et al. 2011; Plett et al. 2014). In the present study, inoculation of *P.*
 413 *trichocarpa* with *M. elongata* (PMI93) was found to alter plant JA signaling,
 414 possibly affecting the suppression of JA-derived pathway. Thus, many
 415 beneficial microorganisms such as *Mortierella* and *Ilyonectria* may use
 416 different strategies to interact with their *Populus* host.

417 *Enrichment of genes for plant lipid pathway in response to PMI93*

418 Given that root-endophytes do not generally grow within plant cells,
 419 interactions between *Populus* and its endophytes must occur within
 420 extracellular spaces. The proportion of genes encoding predicted small-
 421 secreted proteins (pSSPs) for *Populus* in response to *M. elongata* (PMI93) is
 422 high (~40%; 147 genes) compared to other genes for extracellular activities
 423 (Supplementary Datasets 2 and 4). RNA-Seq data indicates that the plant
 424 lipid-transfer proteins (pLTPs) are the primary pSSPs produced by *Populus* in
 425 response to *M. elongata* (PMI93) inoculation (Fig. 6). Only 15 genes for pSSPs
 426 were differentially expressed in *Populus* in response to *I. europaea* (PMI82)
 427 (Supplementary Dataset 4B). In addition, genes for fatty acid/lipid
 428 biosynthetic processes represent the primary set of *Populus* genes enriched
 429 in response to *M. elongata* (PMI93) (Supplementary Fig. 8B), suggesting the
 430 involvement of lipids in *Populus-Mortierella* interactions. As mentioned
 431 above, *Populus* genes for pLTPs were enriched in response to *M. elongata*
 432 (PMI93). These pLTPs contain signal peptides that direct their secretion into
 433 the extracellular matrix. In addition, the affinity of pLTPs for lipids is
 434 presumably fundamental to their function. In other plants, pLTPs have been
 435 shown to be responsible for translocating phospholipids and other fatty acid
 436 groups across cell membranes (Kader 1996). They may also bind to ligands
 437 that contain acyl groups. Ligand prediction analysis indicates these *Populus*
 438 pLTPs can bind to saturated fatty acid (e.g., stearic acid and palmitic acid)
 439 (Supplementary Dataset 4, Supplementary Fig. 5A). The biological roles of

440 pLTPs are still unclear, however several studies suggest their involvement in
 441 antimicrobial activity, defensive signaling, cuticle deposition and cell wall
 442 loosening (Yeats and Rose 2008). Growing evidence also supports another
 443 function of family 1 pLTP to promote plant cell walls extension (Nieuwland et
 444 al. 2005). Up-regulation of gene groups for pLTPs and six expansin-like
 445 proteins and 10 cell wall loosening associated enzymes of *Populus* (Fig. 6;
 446 Supplementary Dataset 4) implicates pLTPs as potential modulators of non-
 447 enzymatic and enzymatic cell wall loosening (Marowa et al. 2016). Similar to
 448 other family 1 pLTPs (Pagnussat et al. 2012), *Populus* pLTPs may function as
 449 the extracellular lipid transfer protein and may be re-localized intracellularly
 450 in order to facilitate fatty acid and lipid-associated pathways.

451 In a separate study, *M. elongata* (PMI93) was observed to produce
 452 polyunsaturated fatty acids under normal growth conditions (Uehling et al.,
 453 2017, Supplementary Fig. 11). The higher number of genes and higher
 454 expression rates of lipid transport and metabolism genes in *M. elongata*
 455 (PMI93) detected in sand-grown *Populus* cuttings compared to cultured
 456 isolates suggests higher induction of *M. elongata* (PMI93) lipid metabolic
 457 activities in the plant-soil system (Supplementary Fig. 9; Supplementary
 458 Dataset 6 and 7). We hypothesize that lipids produced by *Mortierella* may
 459 serve as ligands for pLTPs.

460 *PMI93 manipulates Populus SSP-(pSSPs) and LRR-(pLRR) genes involved in*
 461 *defense responses*

462 In *Populus*, pSSPs for defense responses (Fig. 6) and pLRR-RKs
 463 (Supplementary Dataset 2A; S4), were predominantly down-regulated in
 464 response to *M. elongata* (PMI93). Three groups of these pSSPs were
 465 identified: (a) dirigent-like protein; (b) germin-like protein (c) PR-thaumatin
 466 associated protein (Supplementary Dataset 4). Ligand prediction analysis
 467 showed that these pSSPs are able to bind diverse ligands, implying that
 468 different parts of plant immunity system were suppressed, including JA, SA,
 469 hypersensitive response and LRR-RK associated defense response (see below
 470 explanation).

471 Enzyme active site prediction showed that dirigent-like pSSPs contain
 472 the activity of allene oxide cyclase (AOC) (Supplementary Fig. 5B). These
 473 dirigent-like pSSPs contain an eight-stranded antiparallel beta-barrel with a
 474 central hydrophobic ligand binding site. The predicted ligands of dirigent-like
 475 pSSPs include reaction intermediates required for allene oxide synthase
 476 (AOS) activities (i.e. enoic acid, vernolic acid, Supplementary Fig. 5B)
 477 (Wasternack and Kombrink 2010). The essential function of AOC and AOS in
 478 JA biosynthesis has been reported in other studies (Wasternack 2007; von
 479 Malek et al. 2002; Park et al. 2002; Ishiga et al. 2003), implicating the
 480 manipulation by *M. elongata* (PMI93) in the suppression of JA-mediated plant
 481 defense. Enzyme active site prediction suggests that germin-like pSSPs have
 482 oxalate oxidase (OXO) activity, which can catalyze the conversion between
 483 oxalate and $\text{CO}_2 + \text{H}_2\text{O}_2$. The OXOs are involved in hypersensitive plant cell
 484 death (Lane 2002) and increase their activity under biotic stress (Zhou et al.

1998; Hurkman and Tanaka 1996). Together these results suggest the possibility that *M. elongata* (PMI93) can down-regulate the hypersensitive response of its *Populus* host. Down regulation of several PR-thaumatin associated proteins with beta-1,3-glucanases activity shows that *M. elongata* (PMI93) may suppress multiple routes of plant immunity, including the salicylic acid mediated pathway (Liu et al. 2010) and fungal cell wall degradation (Lusso and Kuć 1996; Kuc 1995).

Plant membrane-localized receptor kinases play important roles in sensing and responding to environmental signals (Osakabe et al. 2013). These receptors perceive the extracellular ligands in order to phosphorylate intracellular kinase domains to activate downstream pathways. Within the *Populus* genome, pLRR-RKs account for the largest group of membrane-localized receptor kinases. pLRR-RKs can exhibit diverse biological functions and most have been shown to play a role in plant defense (McHale et al. 2006). Down regulation of *Populus* pLRR-RKs raises the possibility that *M. elongata* (PMI93) can balance pLRR-RKs signaling and growth tradeoffs to optimize plant fitness.

Conclusions

In conclusion, our findings show differential responses of *Populus* to two functionally different fungal endophytes, *M. elongata* (PMI93) and *I. europaea* (PMI82). These two fungal endophytes have endophytic and saprotrophic activities. The enrichment of a single fungal taxon (PMI93 or PMI82) can shift

507 the whole community of root and soil-associated microbes and can alter the
 508 gene expression of their host plant. RNA-Seq results suggest that *M.*
 509 *elongata* (PMI93) can modulate *Populus* defense responses, nutrient uptake,
 510 and photosynthetic-associated energy production through direct or indirect
 511 interactions with its host. Particularly, the presence of *M. elongata* (PMI93)
 512 leads to downregulation of genes involved in plant immune response and
 513 hormones signaling (e.g., JA/SA/ET signaling, HR response and fungal cell
 514 wall degradation), and alters expression of genes involved in gibberellin and
 515 lipid associated pathways which may result in the observed growth
 516 enhancement. Future studies may consider targeting lipid-derived
 517 communication and metabolism between *Mortierella* and *Populus* roots to
 518 better understand the interactions of these symbionts.

519

520 **Materials and Methods**

521 **Inoculum preparation**

522 Sterile millet seeds were used as the medium to sustain viability and growth
 523 of fungal isolates. To prepare sterile millet seeds for fungal inoculum, millet
 524 seeds were soaked overnight in sterile distilled water. The excess water was
 525 drained, and the wet millet seeds were transferred to mushroom spawn bags
 526 with micropore patch. All bags were tightly closed and autoclaved at 120C,
 527 15 psi for 45 minutes for two cycles and cooled between cycles.

528 Prior to inoculating spawn bags, fungal mycelium [(*M. elongata* (PMI93) and
 529 *I. europaea* (PMI82)] was grown on pure modified Melin Norkrans (MMN) 1%
 530 agar media at 25 °C (Rossi and Oliveira 2011). After 3-days of culturing, the
 531 fungal biomass with solid media was finely chopped into small cubes (around
 532 3x3x3 mm³) and transferred to sterile culture bags containing sterile millet
 533 seeds. After one month of incubation at 25 °C, the fungi had completely
 534 colonized the millet seeds which were used as inoculum in soil bioassay
 535 experiments.

536

537 **Soil bioassay and sample collection for metatranscriptomics**

538 The fresh cuttings of *Populus trichocarpa* were collected from Washington
 539 and Oregon in spring of 2015, including four genotypes: BESC86, GW7974,
 540 BESC4 and BESC320. The 30-cm long vegetative cuttings of *Populus*
 541 *trichocarpa* were submerged in tap water for three days, with daily water
 542 change. Prior to further use cutting were surface sterilized by soaking in 5-
 543 7% bleach solution with 0.01% Tween 20 for 15-20 min and then rinsed with
 544 sterile distilled water. Cuttings were planted into sterile sand and allowed to
 545 root under fluorescent lighting and regular watering. After one month of
 546 growth, the plants were transferred on the same day to 1) soil collected from
 547 forest sites and diluted with sterile sand - soil:sand; 30:70 (w/w), and to 2)
 548 sterile sand only - to serve as “no soil control (sand bioassay)”. Because
 549 fungal inocula grew at different rates, two different fresh soil samples from

the same field site were used for inoculation with *M. elongata* (PMI93) and *I. europaea* (PMI82), respectively. Potted plants were inoculated in replicate with *M. elongata* (PMI93) and *I. europaea* (PMI82), respectively (soil/millet inoculum at 99:1 w:w ratio). Negative control treatments used sterile millet mixed with each soil. Inoculated plants were placed in a growth chamber at 25 °C, 80% humidity and fluorescent light at 200 $\mu\text{mol m}^{-2}\text{s}^{-1}$ for 16 hours per day. After 2-3 months, some replicates of *P. trichocarpa* growing in the sand only without *M. elongata* (PMI93) inoculation did not survive and could not be used for the further studies (Fig.1; Supplementary Fig. 1). After 4 months growth, roots and soils (four biological replicates each) collected from *P. trichocarpa* BESC4 soil bioassay, sand bioassay (with PMI93 inoculum) were harvested for metatranscriptomic analysis. Supplementary Dataset S1 summarized root and soil samples used for metatranscriptomic analysis. Physiological measurements for each plant are presented in Fig. 1 and Supplementary Fig. 1. Additional methods used for soil bioassay are described in Supplementary Fig. 1.

566

567 **RNA preparation, cDNA library construction and Illumina sequencing**

Total RNA from roots and soils were extracted following a CTAB/chloroform extraction and LiCl precipitation protocol previously described (Liao et al. 2014). The mRNA and cDNA for RNA-Seq analysis were purified with a TruSeq RNA sample preparation kit (Illumina, San Diego, CA). cDNA pools

572 were sequenced on the Illumina HiSeq 2000 instruments (Illumina, San
 573 Diego, CA) in the Duke Center for Genomic and Computational Biology
 574 (GCB). Twelve samples were sequenced in an individual lane to generate a
 575 total of ~40Gb of data. In total, 32 samples (root and soils) in soil bioassay, 4
 576 samples in sand bioassay and 3 fungal culture samples were sequenced for
 577 this study. RNA-Seq data have been deposited at NCBI Short Read Archive
 578 (SRP057033).

579

580 **Sequence assembly and annotation**

581 Genome sequences produced by the Joint Genome Institute for *P. trichocarpa*
 582 v3.1 (Tuskan et al. 2006), *Mortierella elongata* AG77 v. 1 (Morel 1) (Uehling
 583 et al. 2017) and *Ilyonectria* (*Ilyonectria europaea* v1.0) were used as
 584 references for RNA-Seq filtered read mapping using Tophat/Cufflink packages
 585 (Trapnell et al. 2010, 2009). The genome and transcriptome of *I. europaea*
 586 were sequenced using Illumina platform. The pipelines applied for the
 587 assembly of *Ilyonectria* genome were described in the Supplementary Text.
 588 Ribosomal rRNA mapping method was employed to sort reads for all other
 589 fungal rRNA as well. Computational workflows for the sequence assembly are
 590 described in Supplementary Fig. 2 (Liao et al., 2014; Liao et al., 2016).
 591 Recovered rRNA reads containing D1/D2 regions were used to calculate
 592 relative abundance of fungal communities (Supplementary Fig. 2). Recent
 593 studies indicate that rRNA reads recovered from metatranscriptome (poly-A

594 enrich strategy) and RNA-based amplicon sequencing detected similar trends
 595 of microbial diversity and community (Liao et al., 2014; Chen et al., 2018).
 596 Nonmetric multidimensional scaling (NMDS) was performed on both
 597 euclidean and bray-curtist dissimilarity matrices, and results from multiple
 598 different dimensions were examined. Results from different configurations
 599 demonstrated similar classification, and results from two dimensions are
 600 presented in Supplementary Figure 3. Differences in community composition
 601 among the treatments (w/o fungal inoculation) were tested using
 602 permutational multivariate analysis of variance (PERMANOVA). Results for
 603 PERMANOVA were corrected for multiple comparison using false discovery
 604 rate (FDR). P-value were calculated based on pseudo-F statistics, and results
 605 with $P \leq 0.05$ were considered as statistically significant (Results shown in
 606 Supplementary Table 2). Both NMDS and PERMANOVA were performed using
 607 vegan package version 2.5.3 in R (3.5.1). They are performed using
 608 metaMDS and adonis functions respectively. Comparative
 609 metatranscriptomics using Cuffdiff and Cuffcompare packages were applied
 610 to identify key plant genes that differentially respond to fungal inoculation
 611 (Trapnell et al. 2010). A false discovery rate (FDR) of 5% was used to identify
 612 highly expressed transcripts with at least 2-fold change for the genes. A
 613 combination of GO (Ashburner et al. 2000), KEGG (Kanehisa et al. 2012) and
 614 KOG (Tatusov et al. 2003) packages was used for gene annotation for *P.*
 615 *trichocarpa* v.3.1. Gene enrichment analysis was applied using the ClueGO
 616 platform (Bindea et al. 2009;2013). Parameters and statistical analysis for

617 gene enrichment analysis were shown in the legend of Supplementary Fig. 8.
 618 It is important to note that only around 18% of the *Populus* genes in the *P.*
 619 *trichocarpa* genomes were assigned to the KEGG and/or GO category. For
 620 example, of 60,000 *P. trichocarpa* genes, 10,876 genes were assigned to
 621 7068 KO numbers. In addition to gene enrichment analysis, several other
 622 software packages were used to better annotate domains to identify the
 623 subcellular locations of genes with an unknown function or predicted with the
 624 extracellular enzymes. For domain analysis, EMBL-EBI, Phobius (Käll et al.
 625 2004), Signal-3L (Zhang and Shen 2017; Shen and Chou 2007) , Signal P v4.1
 626 (Kihara 2017) and TMHMM v2.0 (Krogh et al. 2001) were used for the
 627 prediction of signal-peptides and transmembrane helix domains. ER
 628 retention signal ScanPrositeTool (de Castro et al. 2006) and Euk-mPLoc 2.0
 629 (Chou and Shen 2010) were used to identify the subcellular localization of
 630 contigs. Small secreted proteins were defined as having 1) a size smaller
 631 than 300 amino acids (detected manually), 2) signal peptide predicted at the
 632 N-end (Signal-P v4.1), 3) extracellular location (Euk-mPLoc 2.0), 4) no
 633 transmembrane domains (Euk-mPLoc 2.0, TMHMM v 2.0, EMBL-EBI and
 634 Signal-3L) and 5) no ER retention motifs (ER retention
 635 signal_ScanPrositeTool). The tertiary structures, ligand binding sites and
 636 enzyme activation sites of the individual SSPs were predicted using I-TASSER
 637 v3.0 (Bateman et al. 2002; Zhang 2008; Roy et al. 2010; Yang et al. 2015).
 638 With this approach, we were able to assign the majority of contigs with
 639 unknown function to either small-secreted proteins or transmembrane

640 proteins. Plots (Fig. 4) and heatmaps (Fig. 5 and 6) were generated with
 641 statistical packages in R (R Development Core Team 2003). Given that
 642 different batches of soil were used for *M. elongata* (PMI93) and *I. europaea*
 643 (PMI82) inoculations, we did not attempt to compare the differential
 644 responses of plant between two fungal species. Only the expression of
 645 *Populus* genes that were manipulated by both fungal species are presented
 646 (Fig. 5). The greater number of plant genes responsive to *M. elongata*
 647 (PMI93) were reported in Fig. 6.

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664

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960

961 **Figure legends**

962 **Figure 1.** Plant dry weight (*P. trichocarpa* BESC4) in response to *Mortierella*
 963 *elongata* (PMI93) (A), and *Ilyonectria europaea* (PMI82) (B) inoculation. Two
 964 soil treatments, sterile sand and 30% natural soil (w/ 70% of sterile sand
 965 w/w) collected from *Populus* site (NC1, USA), were used. The data were
 966 collected 1-year post inoculation. Error bars indicate the standard deviation
 967 of data from above-ground tissues (error bars above) and root tissues (error
 968 bar below), n=4. Tukey test was used to test significance of the whole plant
 969 biomass across the combinations ($p \leq 0.05$, n=4). Means marked by the same
 970 letters were not significantly different.

971

972 **Figure 2.** *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) are
 973 transcriptomically active in the forest soil and *Populus* roots and enriched by
 974 inoculation. Bars show the percentage of total RNA-Seq reads of the root and
 975 soil samples (+/- =w/o inoculums) mapped to the genome databases of *M.*
 976 *elongata* and *I. europaea* respectively. Fig. 2A indicates the individual sample
 977 set was mapped to the genome databases of their inoculum, while Fig. 2B
 978 indicates the individual sample set was mapped to the genome databases of
 979 *I. europaea* or *M. elongata*, but not inoculum. The error bars indicate the
 980 standard deviation of the data, n=4. Tukey test was used to test significance

981 of the percentage reads across the combinations in a bar graph ($P \leq 0.05$,
 982 $n=4$). Means marked by the same letters were not significantly different.

983

984 **Figure 3.** Taxonomic composition of fungal communities in uninoculated (-)
 985 root and soil samples and those inoculated (+) with *Mortierella elongata*
 986 (PMI93) and *Ilyonectria europaea* (PMI82). Individual bars show the
 987 normalized abundance of ribosomal RNA sequences (LSU D1D2). Biological
 988 replicates ($n=4$) show a consistent taxonomic representation of reads
 989 recovered from root and soil samples across treatments. Percentages
 990 indicate the relative values of paired reads. The relative values below 1
 991 appear as zero (Over 80% of the relative values <1 were singletons
 992 representatives, data not shown). Taxa are ordered and coded in color based
 993 upon the ecological function of the fungal taxa according to FunGuild
 994 (Nguyen et al. 2016). PERMANOVA were applied to identify the taxa with
 995 significant differential abundance in associated with *Mortierella elongata*
 996 (PMI93) and *Ilyonectria europaea* (PMI82) inoculation. In the right side of the
 997 boxes, the abundance of normalized rRNA reads (using Deseq2) that were
 998 significantly increased (blue boxes) or reduced (red boxes) ($p\text{-value} \leq 0.05$),
 999 or not significantly changed (grey boxes) ($p\text{-value} > 0.05$). The detailed
 1000 statistical results were also shown in Supplementary Table 2 and
 1001 Supplementary Fig. 3.

Figure 4. Volcano plots elucidate the counts and expression rate of *P. trichocarpa* genes up- (green dots) and down- (blue dots) regulated in response to *Mortierella elongata* (PMI93) (A) and *Ilyonectria europaea* (PMI82) (B) inoculation. Black dots represent the expression of genes with no significant difference across the comparisons. Data were generated according to the normalized expression rates using Cufflink packages (see workflow Supplementary Fig. 2). Data of the loading gene factors were generated using the coordinate scales on the left (log10 of expression rate) and the bottom (mean of log2-fold changes). Cross-comparative expression of the genes was analyzed using t-text to compare *P. trichocarpa* with fungal inoculation versus without fungal inoculation ($n=4$; $P \leq 0.01$; $FDR \leq 0.05$; fold changes ≥ 2). The total counts of genes detected and the counts of genes significant changes in response to the inoculation were listed in Supplementary Dataset 2.

Figure 5. *Populus* genes whose expression changes in response to both *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82) inoculation. Significant changes in individual expression between inoculated root (+) v.s. non-inoculated root samples (-) were clustered according to their biological function (>2 -fold changes; $FDR \leq 0.05$, Benjamini-Hochberg test). The color key represents RPKM normalized log2 transformed counts of the genes. Wilcoxon signed-rank test (Bauer, 1972) was applied to filter the data. The

genes for other catalytic activities and unknown function were not included in heatmap (Supplementary Dataset 3). Each *Populus* gene manipulated by the fungal species is given the expression profiles across the four biological replicates per treatment.

Figure 6. Significant changes of *Populus* pSSP genes in response to *Mortierella elongata* (PMI93) inoculation (>2-fold changes; $FDR \leq 0.05$). The method for data analysis see Fig. 5 legend.

Captions for Supplementary Figures

Supplementary Figure 1. (A) Growth enhancement of *Populus trichocarpa* BESC86 in response to inoculation with *Mortierella elongata* PMI93. Without *M. elongata* (PMI93) inoculation, *P. trichocarpa* (BESC86) cuttings did not survive sterile sand conditions. (B) The enhancement of physiological traits (shoot growth, leaf expansion) across 4 different *Populus* genotypes (BESC86, GW7974, BESC4, BESC320) in response to PMI93 inoculation. Soil bioassay (30% soil means 30% soil: 70% sterile sand /w:w) was conducted using natural soil collected from *Populus* field site NC1 (Bonito et al., 2014). (C) Seedling growth and changes in leaf color of *P. trichocarpa* BESC4 in response to PMI93 inoculation. Leaves of inoculated plants were darker green compared to the un-inoculated plants (similar to effect of adding N fertilizer).

1045 **Supplementary Figure 2.** Computational flowchart used to sort the RNA-
 1046 Seq reads of *Populus* root and soil samples. See Dataset S1 for the reads,
 1047 %reads sorted out from each root and soil samples.

1048 **Supplementary Figure 3.** NMDS plots showing the taxonomic composition
 1049 of fungal communities in uninoculated (-) root and soil samples versus those
 1050 inoculated (+) with *Mortierella elongata* (PMI93) and *Ilyonectria europaea*
 1051 (PMI82). PERMANOVA were applied to identify the differential abundance of
 1052 ribosomal RNA sequences (LSU D1D2) in associated with *Mortierella elongata*
 1053 (PMI93) and *Ilyonectria europaea* (PMI82) inoculation. In particular, the
 1054 pairwise comparisons using PERMANOVA were applied to compare the
 1055 uninoculated samples (Group A in green) vs. inoculated samples (Group B in
 1056 orange) across (A) the whole communities and (B) a predicted ecological
 1057 functional group. The statistical outcomes were also shown in Supplementary
 1058 Table 2.

1059 **Supplementary Figure 4.** The combination of sub cellular localization
 1060 analysis and functional annotation show the differential expression of
 1061 *Populus trichocarpa* genes in response to *M. elongata* (PMI93) (A), and *I.*
 1062 *europaea* (PMI82) (B).

1063 **Supplementary Figure 5.** The primary and tertiary (ribbon model) protein
 1064 structures and the predicted ligands of pSSP, include **(A)** lipid transferring
 1065 (pLTPs) and **(B)** defense responsive proteins.

1066 **Supplementary Figure 6.** Key functional categories (KEGG) of *P.*
 1067 *trichocarpa* genes up- (green) and down- (blue) regulated in response to PMI
 1068 93.

1069 **Supplementary Figure 7.** Of 1,055 KOG categorized gene groups detected
 1070 in populus root in response to PMI93 inoculation, top 32 gene groups were
 1071 listed (>2 -fold changes, $FDR \leq 0.05$ for each selected gene; $n=4$).

1072 **Supplementary Figure 8.** The ClueGO pie charts show the enrichment of
 1073 populus genes (% Go terms per group) without (A) versus with PMI93
 1074 inoculation (B) ($p \leq 0.05$).

1075 **Supplementary Figure 9.** (A) Functional proportions of fungal [*M. elongata*
 1076 (PMI93)] genes detected in sand, in culture and presented in *M. elongata*
 1077 genome. (B) Number of *M. elongata* (PMI93) genes significantly expressed
 1078 (>2 -fold changes; $FDR \leq 0.05$) in sand versus in culture system.

1079 **Supplementary Figure 10.** The image observing the mycelium of *M.*
 1080 *elongata* (PMI93) forming the biofilm in associated with corn root (**A**) and
 1081 extending out from the corn root (**B**).

1082

1083 **Supplementary Figure 11.** *Mortierella elongata* (PMI93) growing on MMN
 1084 media producing lipid globules at lipid producing centers (**A** and **B**). Lipid
 1085 release (examples shown by arrow) when the culture is injured with sharp
 1086 object (**C**).

1087 **Captions for Supplementary Table and Text**

1088 **Supplementary Table 1.** The list of *Populus* genes for phytohormone
 1089 biosynthesis/signaling that were responsive to both *Mortierella elongata*
 1090 (PMI93) and *Ilyonectria europaea* (PMI82) vs. either one treatment.

1091 **Supplementary Table 2.** Taxonomic composition of fungal communities in
 1092 uninoculated (-) root and soil samples vs. those inoculated (+) with
 1093 *Mortierella elongata* (PMI93) and *Ilyonectria europaea* (PMI82). PERMANOVA
 1094 were applied to identify the differential abundance of ribosomal RNA
 1095 sequences (LSU D1D2) in associated with *Mortierella elongata* (PMI93) and
 1096 *Ilyonectria europaea* (PMI82) inoculation. In particular, the pairwise
 1097 comparisons using PERMANOVA were applied to compare the uninoculated
 1098 samples vs. inoculated samples across **(A)** the whole communities, **(B)** a
 1099 predicted ecological functional group, or **(C)** each listed fungal taxa.

1100 **Supplementary Text** The pipeline applied for the genome sequencing and
 1101 genome assembly of *Ilyonectria europaea* v1.0

1102

1103 **Captions for Supplementary Dataset**

1104 **Supplementary Dataset S1.** Reads and %reads of fungal D1D2 LSU rRNA,
 1105 fungal genes and plant genes sorted from root and soil samples using
 1106 computational workflow (Supplementary Fig. 5)

1107 **Supplementary Dataset S2.** *Populus* gene significantly changes in
 1108 response to *M. elongata* (PMI93) and *I. europaea* (PMI82) inoculation
 1109 (FDR \leq 0.05; >2-fold; n=4) (S10_9to12 versus S11_1to4). The data of
 1110 Supplementary Dataset S2 was used to generate Volcano plots in Fig 4.

1111 **Supplementary Dataset S3.** The list of shared genes of *P. trichocarpa* in
 1112 response to *M. elongata* (PMI93) and *I. europaea* (PMI82). The data of
 1113 Supplementary Dataset S3 was used to generate heatmap for Fig 5.

1114 **Supplementary Dataset S4.** Differential expression of pSSP-like genes of
 1115 *P. trichocarpa* in response to PMI93 (Dataset S4A, 147 genes) and PMI82
 1116 (Dataset S4B, 15 genes) respectively. The data of Supplementary Dataset S4
 1117 was used to generate heatmap for Fig 6.

1118 **Supplementary Dataset S5.** Gene products (7950 transcripts) of *M.*
 1119 *elongata* (PMI93) detected in sand with *P. trichocarpa* BESC4 grown nearby
 1120 (n=4).

1121 **Supplementary Dataset S6.** Gene counts and % of gene counts of *M.*
 1122 *elongata* (PMI93) detected in sand with *P. trichocarpa* BESC4 grown nearby
 1123 (n=4), culture condition and presented in Morel1 genome. The data of
 1124 Supplementary Dataset S7 was used to generate bar graph of Fig. S8A.

1125 **Supplementary Dataset S7.** Comparative transcriptomic data showing the
 1126 differential expression of *M. elongata* (PMI93) genes in sand condition (with

1127 *P. trichocarpa* BESC4 grown nearby) verse culture conditions. The data of
1128 Supplementary Dataset S7 was used to generate bar graph of Fig. S8B.
1129 **Supplementary Dataset S8.** The list of fungal small secreted protein
1130 (fSSP) predicted in *Mortierella elongata* (Morel1) genome.